Supporting information for

A near-infrared Nile Red fluorescence probe for discrimination of biothiols by dual-channel response and its bioimaging applications in living cells and animals

Jin-Shuai Lan,[a†] Rui-Feng Zeng,[b†] Yun Liu,[a] Yan-Wei Xiang,[c] Xiao-yi Jiang,[b] Li Liu,[b] Sai-Sai Xie,[d] Yue Ding,[a*] Tong Zhang[a*]

[a] *Experiment Center of Teaching & Learning, Shanghai University of Traditional Chinese Medicine, Shanghai, China;*

[b] *School of Pharmacy, Shanghai University of Traditional Chinese Medicine, Shanghai, China;*

[c] *Headmaster's office, Shanghai University of Traditional Chinese Medicine, Shanghai, China.*

[d] *National Pharmaceutical Engineering Center for Solid Preparation in Chinese Herbal Medicine, Jiangxi University of Traditional Chinese Medicine, Nanchang 330006, China*

*****Correspondence: zhangtongshutcm@hotmail.com (T.Z.); dingyue-2001@hotmail.com (Y.D.);

Tel.: +86-021-51322318 (T.Z.)

[†] These authors contributed equally to this work.

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Figure S2. Fluorescence spectra changes of the probe NR-NBD upon the addition of Na_2S and GSH.

Figure S3. The fluorescence emission intensity changes of NR-NBD in the presence of Cys under a constant irradiation within 2 hour in DMSO: PBS solution.

Figure S4. Effect of pH on the fluorescence intensity of the probe NR-NBD.

Figure S5. Fluorescence intensity of the probe NR-NBD in DMSO: PBS solution upon addition addition of Cys, Hcy, GSH, H_2S and different amino acid.

Figure S6. HPLC chromatogram of probe in the reaction with Cys, Hcy, GSH and H_2S in DMSO/PBS solution.

Figure S7. Mass spectrum of the crude product from the probe NR-NBD, Nile-Red, and the reaction of the probe NR-NBD with Cys, Hcy, GSH and H_2S.

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Figure S10. ^{1}H-NMR spectra of the probe NR-NBD in $CDCl_3$: MeOD (20: 1, v:v).

Figure S11. ^{13}C-NMR spectra of the probe NR-NBD in $CDCl_3$: MeOD (1: 3, v: v).

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Table S1. The comparison data with some other fluorescent probes

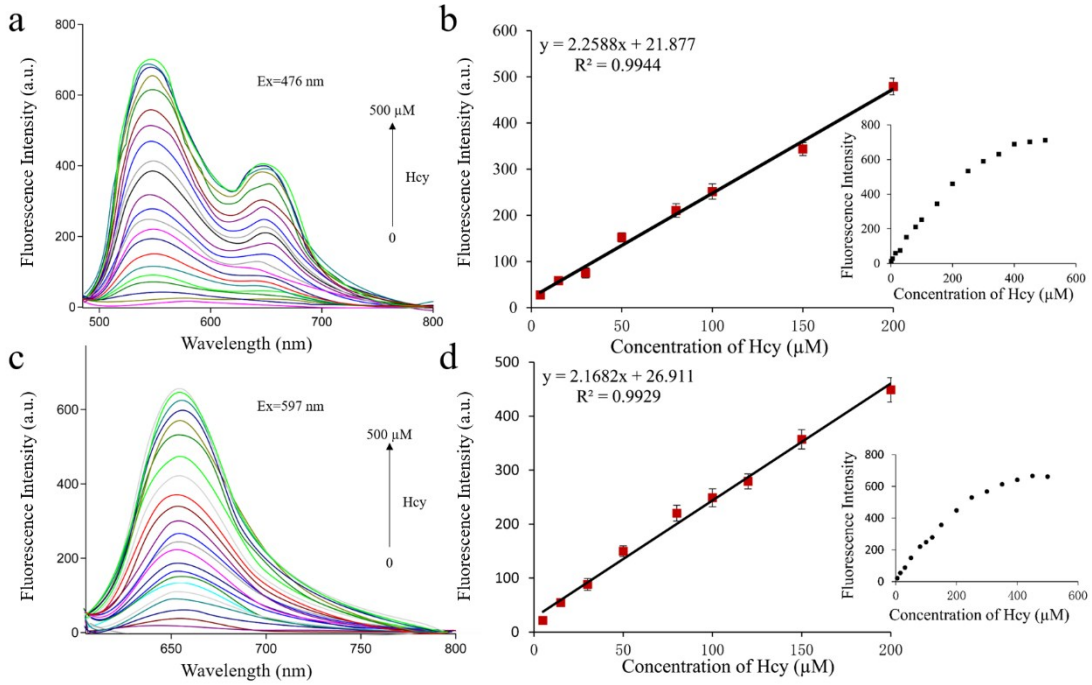


Figure S1. (a-d) fluorescence spectra changes of the probe NR-NBD (10 μM) upon addition of increasing amount of Hcy (0-500 μM). For (a-b) λ_{ex} = 476 nm, for (c-d) λ_{ex} = 597 nm. Slits: 10.0/5.0 nm.

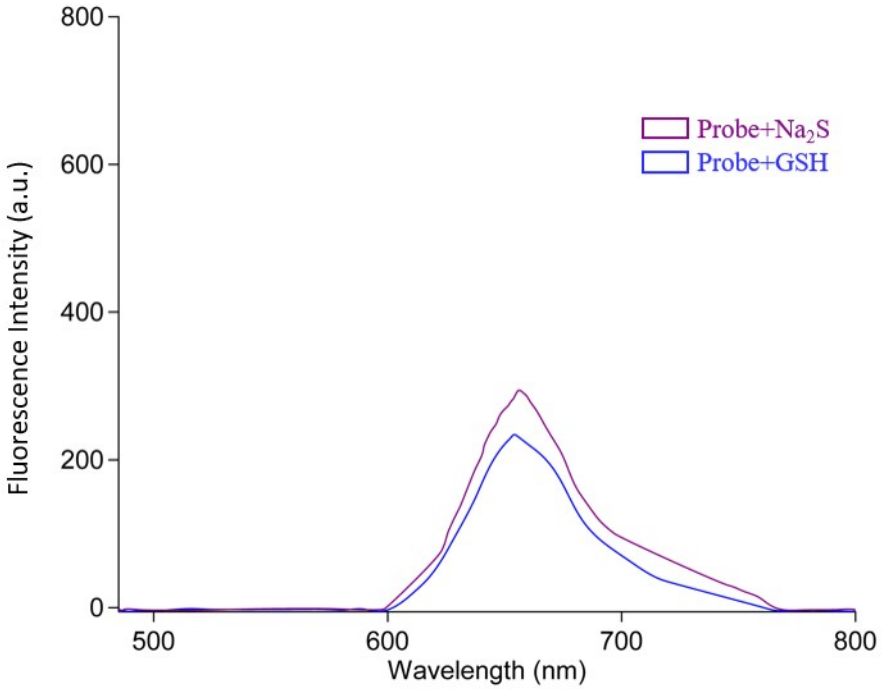


Figure S2. Fluorescence spectra changes of the probe NR-NBD (10 μM) upon the addition of Na_2S (500 μM) and GSH (500 μM). For λ_{ex} = 476 nm. Slits: 10.0/5.0 nm.

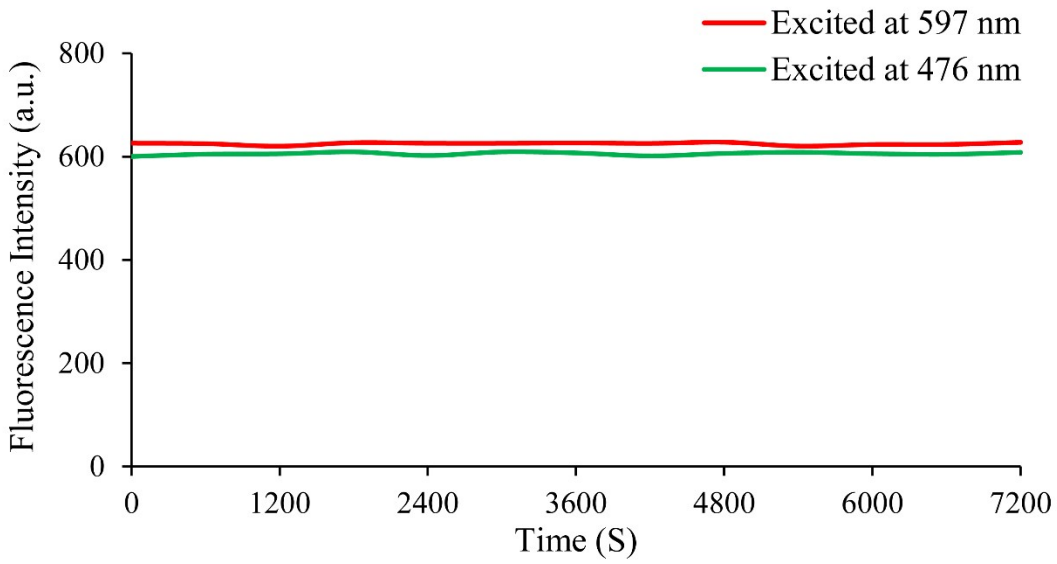


Figure S3. After complete reaction, the fluorescence emission intensity changes of NR-NBD (10 μM) in the presence of Cys (500 μM) under a constant irradiation within 2 hour in DMSO: PBS solution (4: 6, v/v, pH = 7.4).

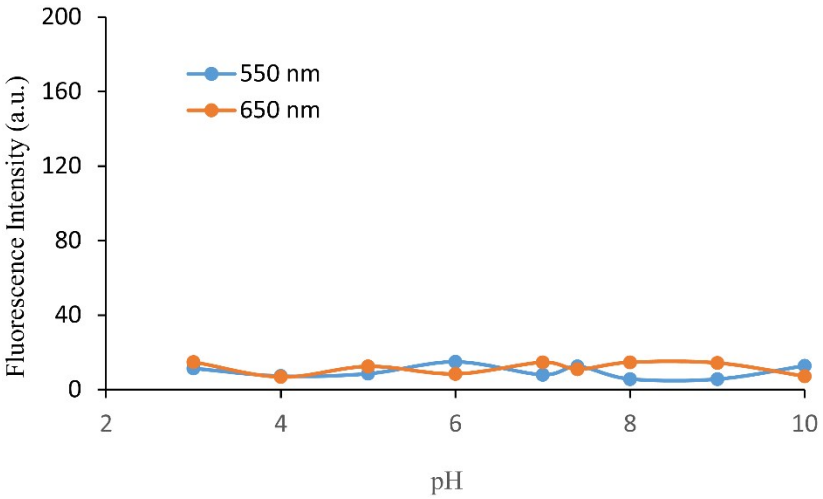


Figure S4. Effect of pH on the fluorescence intensity of the probe NR-NBD (10 μM). For λ_{ex} = 476 nm, λ_{em} = 550 nm; λ_{ex} = 597 nm, λ_{em} = 650 nm. Slits: 10.0/5.0 nm.

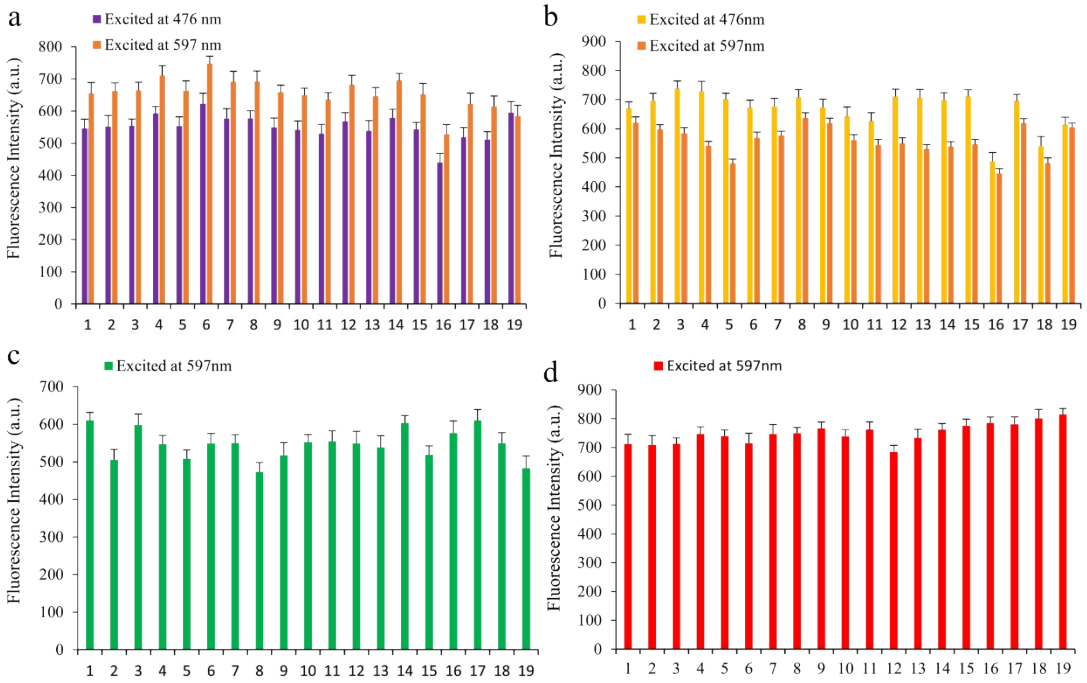


Figure S5. Fluorescence intensity of the probe NR-NBD (10 μM) in DMSO:PBS solution (4:6, v/v, pH=7.4) upon addition a. Cys, b. Hcy, c. GSH, d. Na_2S (500 μM) and various other anions. 0.blank, 1. Tyrosine (Try), 2. Lysine (Lys), 3. Alanine (Ala), 4. Histidine (His), 5. Glycine (Gly), 6. Arginine (Arg), 7. Glutamine (Glu), 8. Cystine (Cys C), 9. Serine (Ser), 10. Methionine (Met), 11. Valine (Val), 12. Proline (Pro), 13. Threonine (Thr), 14. Isoleucine (Iso), 15. Leucine (Leu), 16. Aspartic acid (Asp), 17. Phenylalanine (Phe), 18. Glutamic acid (Glu), 19. None. For (a) λ_{ex} = 476 nm, for (b) λ_{ex} = 597 nm. Slits: 10.0/5.0 nm.

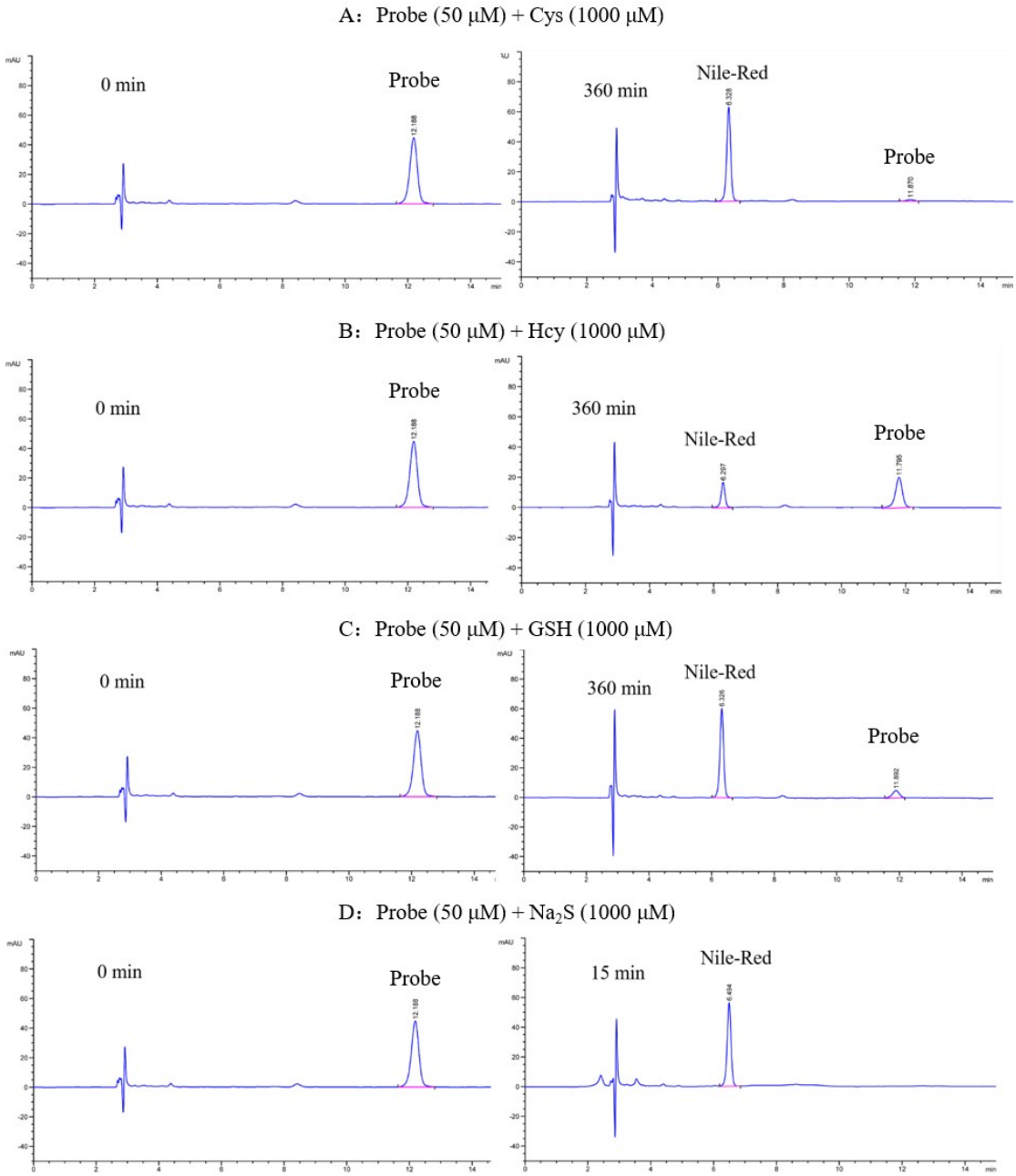
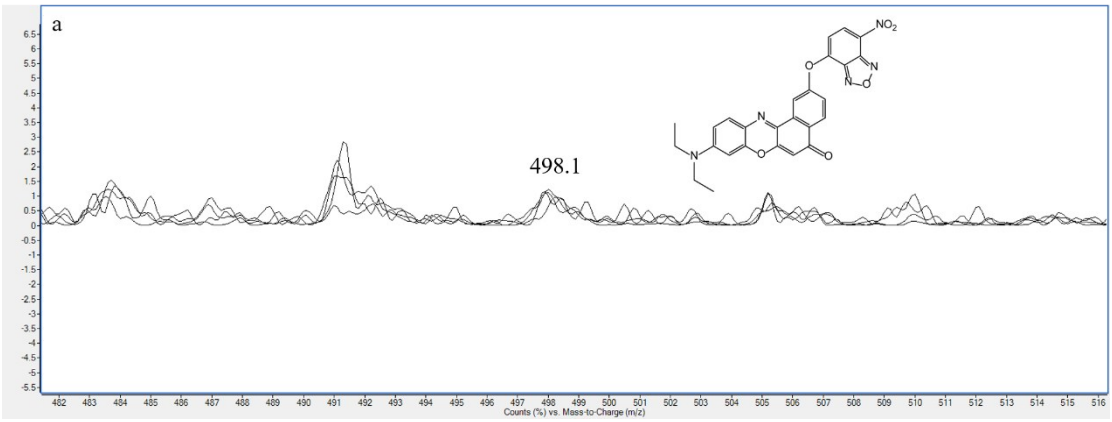
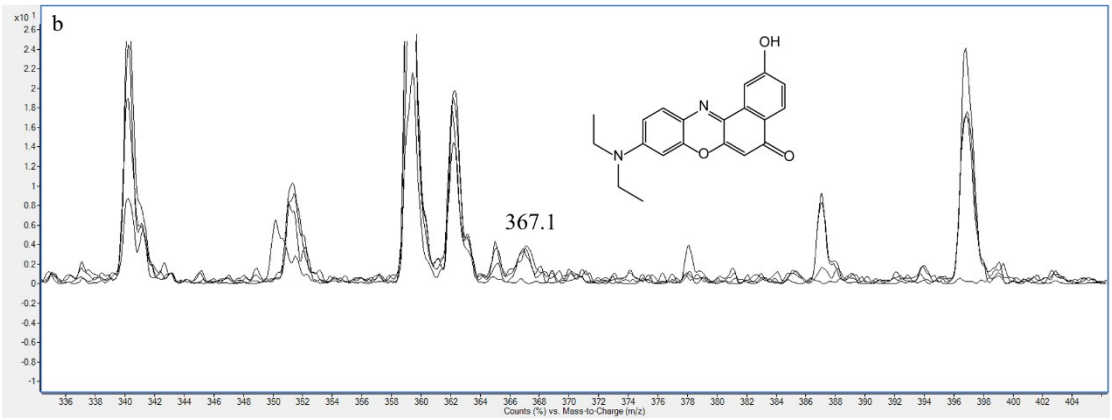


Figure S6. HPLC chromatogram of probe (50 μM) in the reaction with biothiols (20 equiv.) in DMSO/PBS solution (4: 6, v/v, pH = 7.4).

HPLC method: An HPLC system (Agilent Technologies 1260-series, Agilent, USA), with a quaternary pump and a UV-DAD detector equipped with a C18 column (250 mm × 4.6 mm, internal diameter 5 μm, Zorbax Eclipse Plus, Agilent, USA), was used. Chromatography was performed with H_2O:MeOH-15:85 and the flow rate of the mobile phase was 1.0 ml/min. 10 μl of the sample was injected. The column was

purged with the mobile phase for 10 min, followed by equilibration for 20 min, and then 20 min were required for sample analysis at 25 ℃. Spectral data were collected at detection wavelengths of 254 nm.


a
498.1
Counts (%) vs. Mass-to-Charge (m/z)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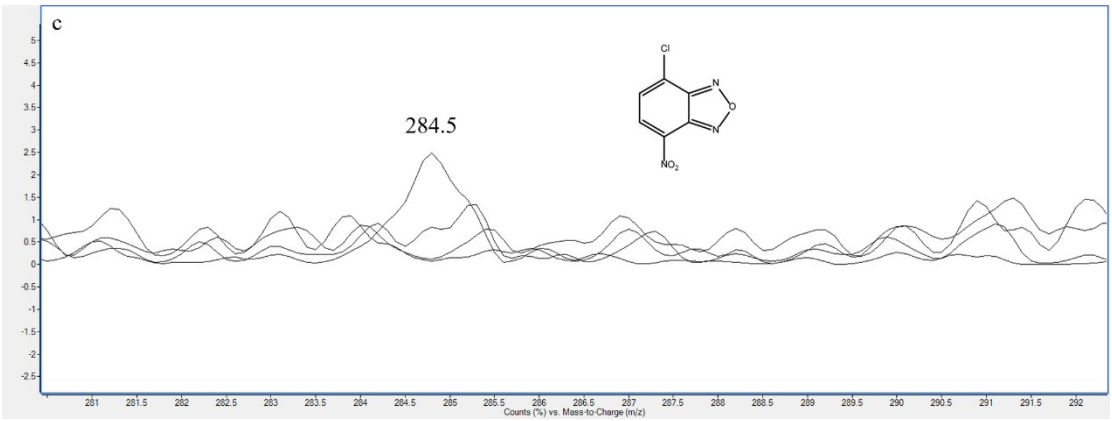
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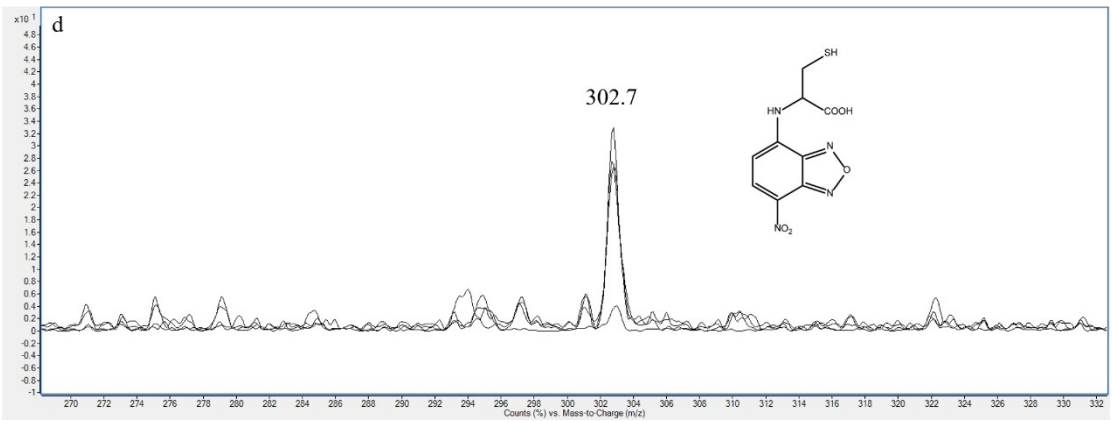
284.5
Counts (%) vs. Mass-to-Charge (m/z)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Counts (%) vs. Mass-to-Charge (m/z)

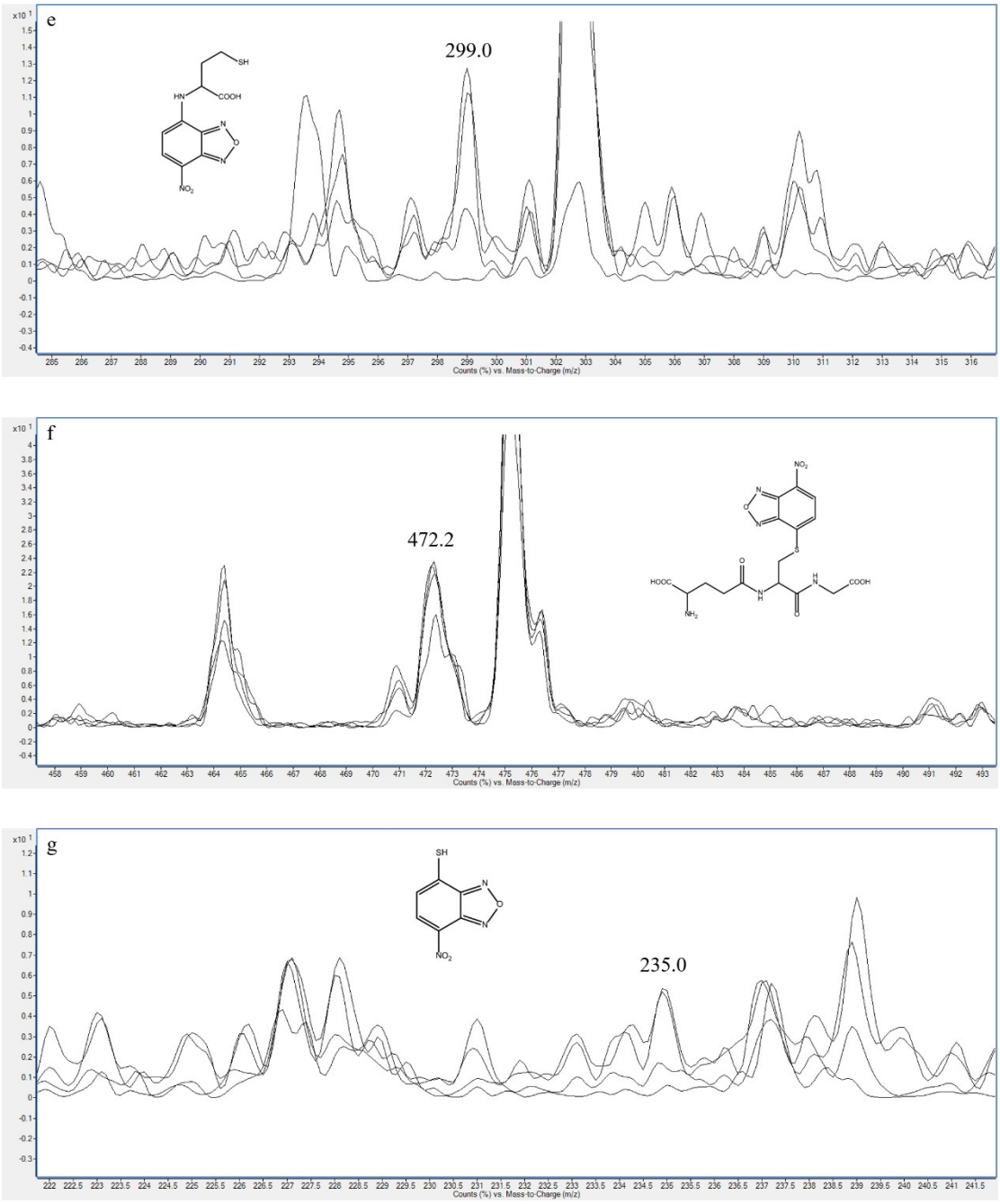


Figure S7. Mass spectrum of the crude product from (a) the probe NR-NBD, (b) Nile-Red, (c) NBD and the reaction of the probe NR-NBD (d) with Cys, (e) with Hcy, (f) with GSH, (g) with H_2S.

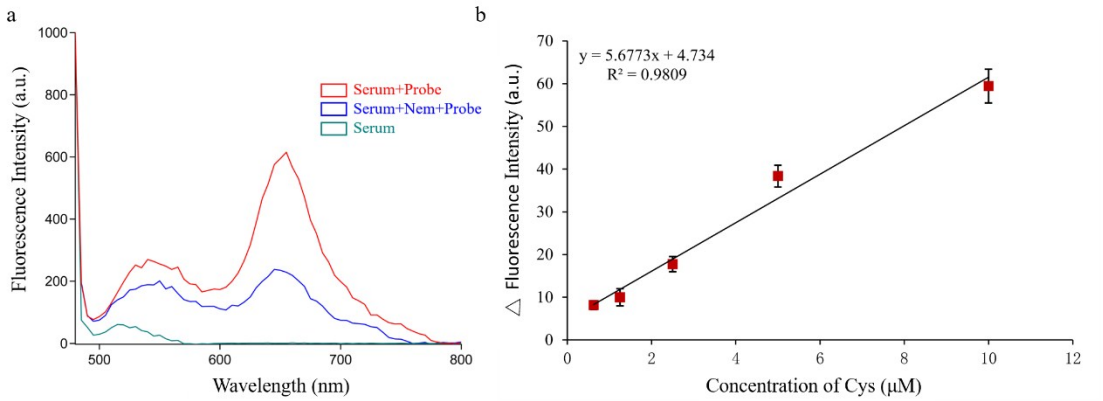


Figure S8. (a) Fluorescence intensity of the probe NR-NBD (10 μM) in DMSO/PBS solution (4: 6, v/v, pH = 7.4) upon addition of various serum samples (2%), λ_{ex} = 476 nm, Slits: 10.0/5.0 nm. (b) Working curves of probe NR-NBD (10 μM) to Cys in normal serum samples.

Human serum (100 uL) was deproteinized using Methanol (3 mL) containing 1% acetic acid and centrifuging at 15000 rpm for 10 min. The supernatant was blow-dried and diluted in 5 mL DMSO/PBS solution (4: 6, v/v, pH = 7.4). The Cys contents in the serum sample were determined using the same procedure above and the standard calibration curves.

To build a standard curve, we firstly measured the fluorescence spectra of 2% serum in the absence/presence the probe NR-NBD (10 μM). As we known, N-ethylmaleimide (NEM) was a specific inhibitor for thiols including Cys. Compared with the group of probe + serum, the fluorescence intensity of probe + serum + NEM at 550 nm was decreased because the Cys was consumed by NEM. Then Cys is quantified by the difference between group probe + serum and group probe + serum + NEM at 550 nm. By plotting the fluorescence intensity versus the concentrations of analytes, a standard curve was obtained, as shown in Fig.S8.

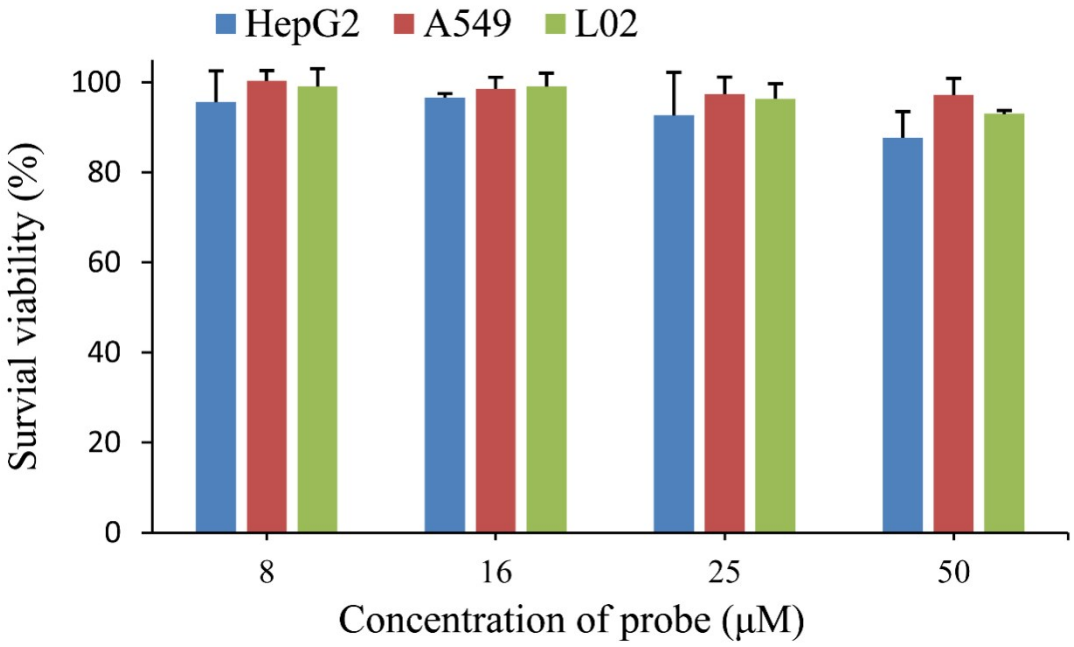


Figure S9. CCK8 assay of (a) HepG2, (b) A549, (c) L02 cells incubated in the presence of the probe NR-NBD (0-50 μM) at 37 ℃ for 24 h.

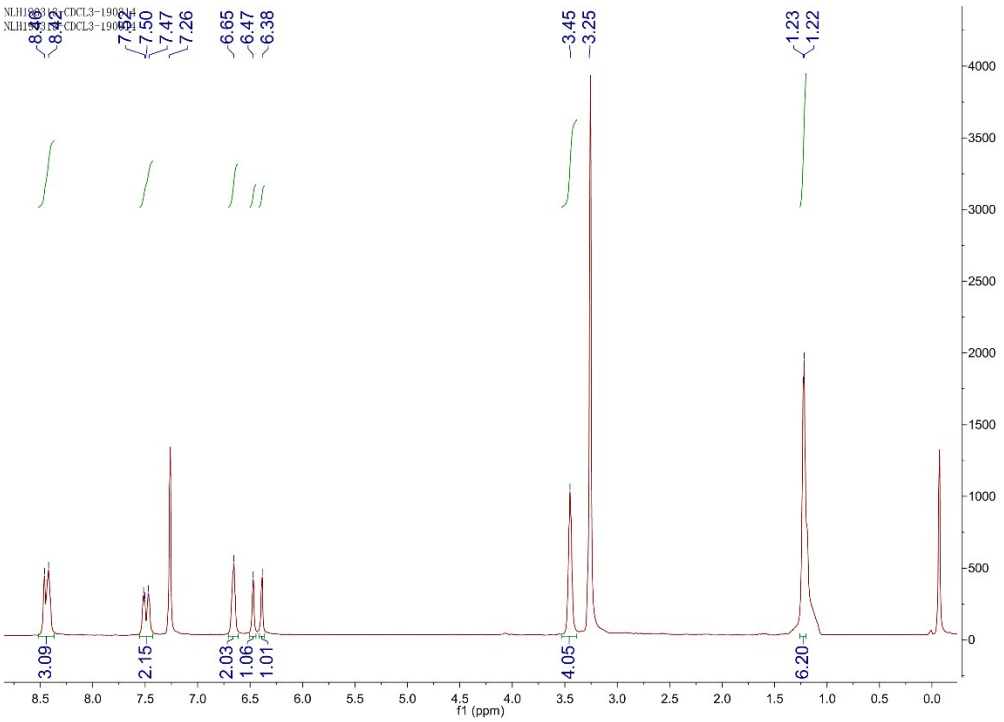


Figure S10. ^{1}H-NMR spectra of the probe NR-NBD in $CDCl_3$: MeOD (20:1, v:v).

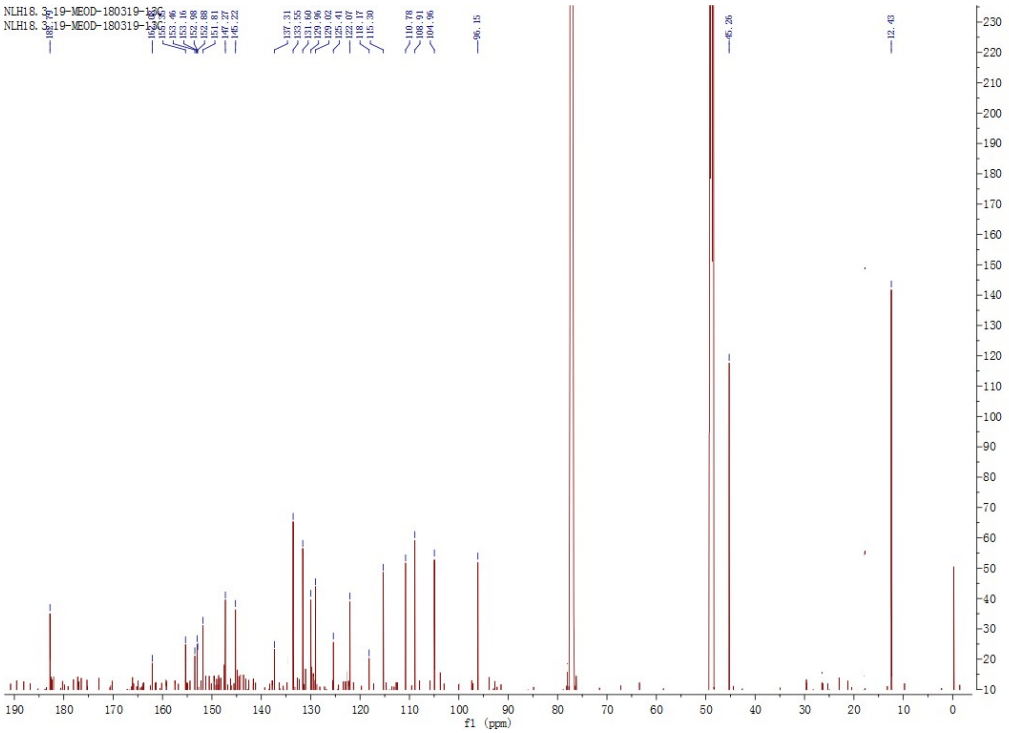


Figure S11. ^{13}C-NMR spectra of the probe NR-NBD in $CDCl_3$: MeOD (1:3, v:v).

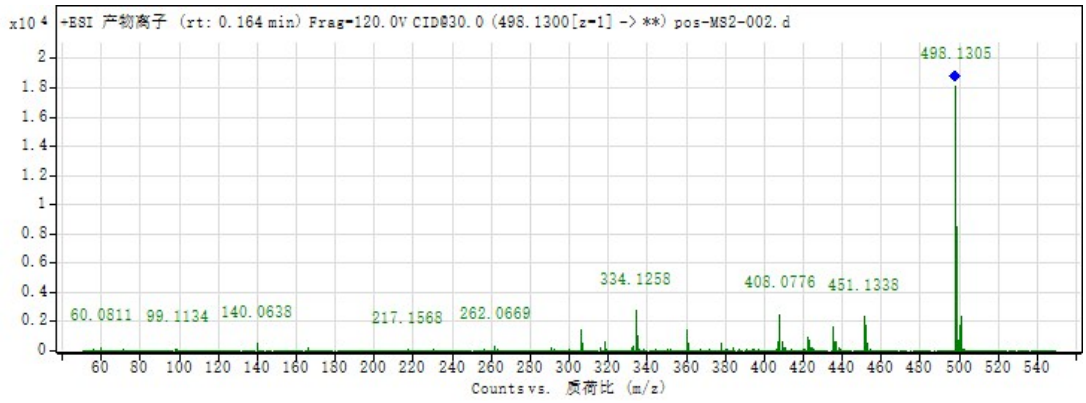


Figure S12. HRMS spectrum of probe NR-NBD in MeOH.

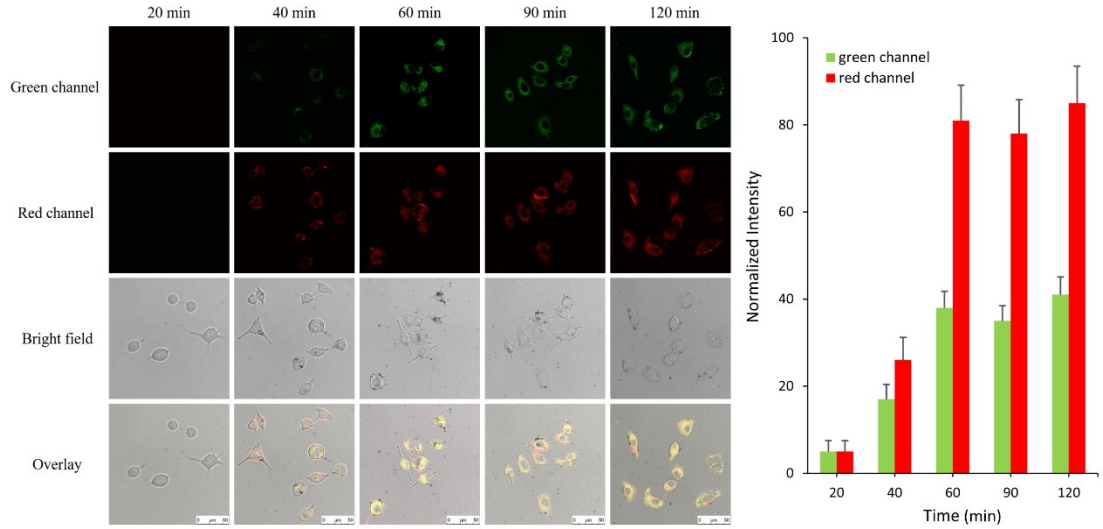
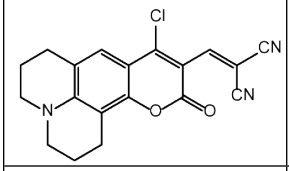
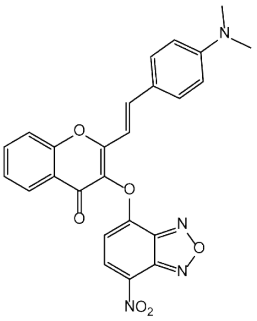
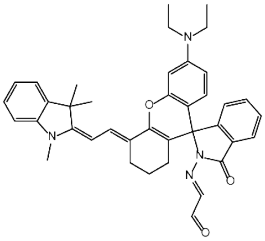
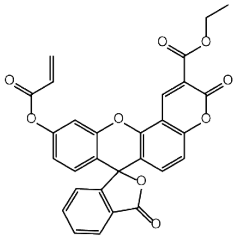
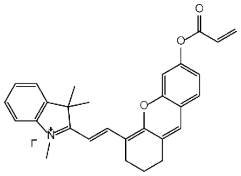
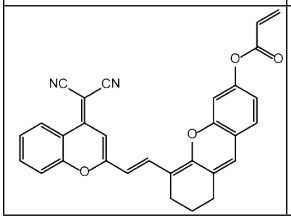
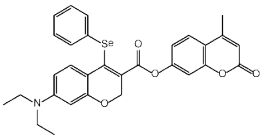
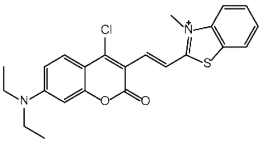
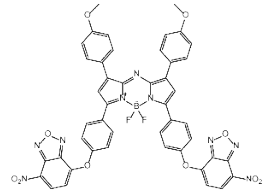
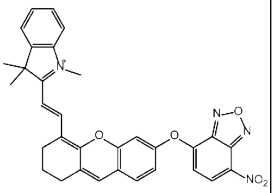
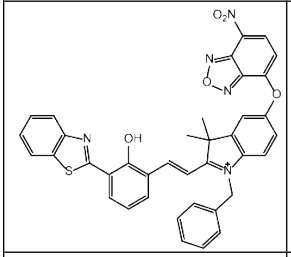
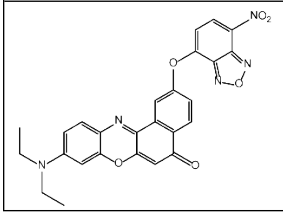


Figure S13. The fluorescence intensity of probe NR-NBD (20 μM) with different incubated time in living cells pretreated with Cys (500 μM). The cells were incubated with probe NR-NBD (20 μM) for (a) 20 min. (b) 40 min. (c) 60 min. (d) 90 min. (e) 120 min.

Table S1. The comparison data with some other fluorescent probes

Multisite probe	Distinguishing targets for detection	Number of emission bands	Emission wavelength/nm	biological system	Ref.
	Cys/Hcy, GSH	2	500, 560	HeLa cells	[1]
	Cys/Hcy,GSH	2	545,621	HeLa cells	[2]
	GSH	1	735	HeLa cells	[3]
	Cys	2	332, 450	HepG2 cells	[4]
	Cys	1	679	HeLa cells	[5]
	Cys	1	760	HeLa cells Mice	[6]

	Cys/Hcy, GSH/H_2S	2	465,540	RAW264.7 cells	[7]
	Cys, GSH	2	420, 512	COS-7 cells	[8]
	Cys/Hcy, GSH	2	540/730, 730	HeLa cells	[9]
	Cys/Hcy, GSH	2	550, 716	HeLa cells, human serum	[10]
	H_2S, Cys/Hcy, GSH	3	485, 546, 609	HeLa cells	[11]
	H_2S, Cys/Hcy, GSH	2	550, 650	HepG2 cells A549 cells, L02 cells, mice serum, mice	This work

Reference

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